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ICMSF methods studies. I. Comparison of analytical schemes for detection of *Salmonella* in dried foods[1,2,3]

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Twenty-six low-moisture food samples naturally contaminated with salmonellae were analyzed to compare the efficiency of sixty 25-g, fifteen 100-g, and three 500-g subsamples in recovery of salmonellae. In addition, the efficacy of wet compositing in recovery of salmonellae was studied by pooling in groups of five, the pre-enrichment broths from the 25-g and 100-g subsamples. Eighteen of the samples were positive by all sampling procedures. Four of the samples were negative when sixty 25-g subsamples were analyzed, four were negative by the fifteen 100-g sub-samples, and two were negative by the three 500-g subsamples. Wet-compositing results indicate the method is as sensitive in detecting salmonellae as the analysis of individual subsamples. The overall results indicate samonellae may be detected with equal facility by any of the sampling and analytical methods tested.

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On a analysé vingt-six échantillons d'aliments, ayant un taux peu élevé d'humidité et contaminés naturellement par des salmonelles. Le but de cette étude était de comparer l'efficacité de 60 sous-échantillons de 25 g, 15 de 100 g et 3 de 500 g pour la récupération des salmonelles. De plus, on a étudié l'efficacité d'un mélange mouillé dans la récupération des salmonelles en répartissant, par groupes de cinq, les bouillons de pré-enrichissement de sous-échantillons de 25 et 100 g. Quelle que soit la méthode d'échantillonnage; dix-huit des échantillons ont été positifs, quatre furent négatifs dans l'analyse de 60 sous-échantillons de 25 g; quatre furent également négatifs pour 15 sous-échantillons de 100 g et, finalement deux furent négatifs dans l'analyse de 3 sous-échantillons de 500 g. Cette méthode des mélanges mouillés s'est avérée aussi efficace dans la détection des salmonelles que l'analyse des sous-échantillons individuels. Dans leur ensemble les résultats démontrent que les salmonelles peuvent être facilement décelées quelles que soient les méthodes d'analyses ou d'échantillonnages utilisées.
[Traduit par le journal]

The sensitivity of the analytical methods is most important in the design of *Salmonella* control systems for foods. Increasing the amount of sample examined from a particular lot decreases the probability of accepting a positive lot. There has been a trend to analyzing increasingly larger samples, but the sensitivity of detecting *Salmonella* in large samples has not been adequately determined. The testing of 60 individual 25-g subsamples with negative results was recommended by the National Research Council (NRC) Committee on *Salmonella* (2) before high-risk foods could be released for consumption by aged and infirm persons and infants. It is obvious that following this recommendation would not be economically feasible on a routine basis. Examination of fifteen 100-g subsamples would require one-fourth the laboratory work but would be warranted only if the sampling and analytical scheme were as efficient in detecting positive lots as one involving the analysis of sixty 25-g subsamples. Similarly, if an analytical method using three 500-g subsamples from the lot were equally capable of detecting contamination, then the laboratory cost of statistical quality control over *Salmonella* would be greatly reduced, and more widespread application of statistical control programs would be encouraged.

Compositing of multiple subsamples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control. Both approaches are being used widely by the food industry in the United States, but little has been published on the efficacy of either technique.

Silliker (appendix C of reference 2) examined 389 lots of dried egg products by analyzing

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400-g composite samples as well as multiple 25-g samples. His data suggested some loss in sensitivity caused by the use of 400-g samples. Huhtanen *et al.* (1) analyzed 25 meat-and-bone meal samples for salmonellae, conparing a single 300-g sample with ten 30-g samples. Seventeen were positive on the testing of a single 300-g sample; 18 were positive on the testing of ten 30-g samples.

Silliker (appendix D of reference 2) reported that of 106 wet composites derived from 1060 individual samples, 39 were positive for *Salmonella* and 66 were negative. In seven instances negative results were obtained despite the fact that one or more of the 10 individual samples comprising the wet composite did contain salmonellae. Thus, these seven were false negative results. Price *et al.* (3) reported that as many as 25 pre-enrichment broth cultures could be pooled without apparent loss in sensitivity.

The present work compares five analytical systems for the detection of salmonellae in dried foods. In each system 1500 g of sample was tested. The studies were designed to compare the following analytical schemes: (1) Sixty 25-g subsamples; (2) fifteen 100-g subsamples; (3) three 500-g subsamples; (4) wet compositing of 25-g pre-enrichment cultures in groups of five; and (5) wet compositing of 100-g pre-enrichment cultures in groups of five. The work is part of a broad study program of analytical microbiological methods for foods being conducted by the International Commission on Microbiological Specifications for Foods.

Methods

Source of Samples

All test samples were commercial products, believed to be naturally contaminated with salmonellae. Each sample comprised 70–80 kg; the major portion of this was consumed in primary testing.

Sample Preparation

Certain highly contaminated samples were diluted with *Salmonella*-free material of the same composition, as shown in Table 1.

Chocolate-almond bars and milk chocolate were ground in a meat grinder through a plate with holes 8 mm in diameter, then hand-mixed and reground through a plate with holes 3 mm in diameter. The other foods (with exceptions noted below) were mixed for 1 h in a stainless steel liquid–solids blendor (Patterson-Kelley Company, Inc., East Stroudsburg, Pa.).

The fish meal used in test 22 and the coconut used in test 25 were not mixed. In test 26 contaminated coconut was diluted 1:3 with uncontaminated coconut, mixed by hand, and then ground twice in the meat grinder through a plate containing holes 3 mm in diameter.

Sampling

In tests 1–21 the mixed products were sampled directly, i.e., sixty 25-g, fifteen 100-g, and three 500-g subsamples were weighed directly into the pre-enrichment broth. In tests 22–26, 180 25-g subsamples were weighed into 180 sterile sample bottles. Sixty of the 180 subsamples were randomly selected for the 25-g subsamples. The 100-g subsamples were prepared by randomly pooling four 25-g subsamples to form 15 groups. The 500-g subsamples were prepared by randomly pooling groups of twenty 25-g subsamples.

Pre-enrichment

Chocolate-almond and milk-chocolate samples were enriched in reconstituted 10% (w/v) non-fat dry milk containing 0.002% brilliant green dye. Black pepper, whole egg solids, egg yolk solids, egg albumen, and isolated soy protein samples were enriched in lactose broth (Difco, Detroit, Michigan). Meat meal, cottonseed meal, fish meal, and desiccated coconut samples were enriched in lactose broth containing 1% (v/v) Tergitol Anionic 7 (sodium heptadecyl sulfate, Union Carbide Company, Chicago, Illinois).

The 25-g subsamples were enriched in 225 ml of broth contained in 500-ml jars with screw-cap lids. The 100-g subsamples were enriched in 900 ml of broth contained in 1500-ml bottles with screw-cap lids. The 500-g subsamples were enriched in 4500 ml of broth in 8-liter polyethylene containers (Rubber-Maid Company, Commercial Division, Winchester, Va.). The containers were covered with aluminum pie pans with 20-cm diameters. Since the polyethylene containers were not resistant to 121C, all media were pasteurized in flowing steam for 20 min. The temperature of the media after this treatment was greater than 85C. The media were used immediately after cooling to room temperature.

Subculturing of Pre-enrichment Cultures

All pre-enrichment cultures were incubated for 24 h at 35C. After incubation, 1 ml from each of the cultures was introduced into 9 ml selenite–cystine broth (Difco) and 9 ml tetrathionate broth containing brilliant green dye (Difco) (4).

Sixty 25-g pre-enrichment cultures were randomly assigned to 12 groups of five. Each of these groups was used to prepare wet composites as follows: 1 ml from each of the five pre-enrichment cultures was introduced both into 45 ml selenite broth and 45 ml tetrathionate broth. Similarly, the fifteen 100-g pre-enrichment cultures were randomized and assigned to three groups of five. Wet composites of the 100-g pre-enrichment cultures were prepared in the same manner as described above. The selenite and tetrathionate subcultures were incubated 24 h at 35C.

Selective and Differential Media

After incubation, each of the selenite and tetrathionate broth cultures was streaked onto brilliant green (BG), *Salmonella–Shigella* (SS), and bismuth sulfite (BS) agars (Difco) and incubated at 35C. The SS and BG plates were examined after 24 h, the BS plates after 48 h.

Identification

Suspect colonies were identified by conventional biochemical and serological procedures (4). The serological identification included slide agglutinations using group specific O antisera (Difco) and tube agglutinations using Spicer-Edwards H antisera (Difco).

Results

Eighteen of the 26 samples were positive for salmonellae by all analytical procedures (Table 1). Four of the samples were negative when sixty 25-g subsamples were used, but the same samples were positive by one or more of the other analytical procedures. The twelve 25-g wet composites derived from each of these four samples were also negative. Four of the test samples were negative in the fifteen 100-g subsamples, and the wet composites derived from these aliquots were likewise negative. In test 18, three of the fifteen 100-g subsamples were positive, but all of the wet composites were negative. A minimum of one and a maximum of three positives would be expected from the wet composites; thus one or more of the negative results on the 100-g wet composites in test 18 must be considered as "false negative." Noteworthy was the detection of *Salmonella* in 24 of 26 test samples when three 500-g subsamples from each test sample were studied.

Table 1 indicates that the failure of one or more of the analytical procedures to detect salmonellae was generally associated with samples containing a low level of contamination.

TABLE 1

Comparison of analytical methods for detection of salmonellae in dried foods

		Salmonella-positive results				
		Dry subsamples			Wet composites	
		Wt. of aliquots, g				
		25	100	500	25	100
		No. of aliquots				
Test	Product	60	15	3	12	3
1	Chocolate almond bar	59	15	3	12	3
2	As test 1 diluted 1:10	39	13	3	12	3
3	As test 1 diluted 1:40	9	5	3	6	2
4	As test 1 diluted 1:100	3	2	0	2	1
23	As test 1 diluted 1:40	5	2	1	2	1
5	Milk chocolate	0	0	1	0	0
6	Black pepper	0	1	1	0	1
7	Whole egg solids	0	1	1	0	1
8	Egg yolk solids	2	1	1	2	1
9	Egg albumen solids lot 1	59	14	3	12	3
10	Egg albumen solids lot 2	60	15	3	12	3
12	As test 10 diluted 1:2.6	45	13	3	11	3
13	As test 10 diluted 1:26	2	0	0	2	0
11	Mixture albumen solids lots 1 and 2 diluted 1:4	56	15	3	12	3
14	Isolated soy protein	0	0	1	0	0
15	Meat meal lot 1	28	15	2	11	3
17	As test 15 diluted 1:4	4	0	1	4	0
16	Meat meal lot 2	6	2	1	4	1
21	Meat meal lot 3	58	15	3	12	3
18	Cottonseed meal	7	3	2	4	0
19	Fish meal lot 1	22	10	3	8	2
20	Fish meal lot 2	20	11	3	11	3
22	Fish meal lot 3	60	15	3	No test	No test
24	As test 22 diluted 1:100	10	11	3	4	3
25	Desiccated coconut	43	15	3	11	1
26	As test 25 diluted 1:3	2	3	1	2	2

By assuming that the contamination in samples negative by one or more of the methods was sufficiently low so that a single positive resulted from a single *Salmonella*, then the number of salmonellae per 500 grams may be estimated.

Table 2 represents in condensed form the patterns of salmonellae recovery from the 26 samples and the estimated level of contamination in samples negative by one or more methods. The estimation of the *Salmonella* contamination was derived by calculation. For example, in test 4 a total of five positives were obtained from the sixty 25-g, fifteen 100-g, and three 500-g subsamples. Assuming that any positive resulted from a single *Salmonella*, then, in the total of 4500 g of sample analyzed (wet composites not included), there were five salmonellae. The best estimate of contamination in 500 g would be five positives in 4500 g divided by nine, or 0.56 salmonellae per 500 grams. Estimates of the number of salmonellae per 500 grams have been calculated for each lot found negative by one or more of the five analytical procedures.

The estimates of the level of contamination are in the right-hand column of Table 2. In seven of the eight test samples found negative by any one of the analytical schemes, the level of salmonellae was calculated to be less than one *Salmonella* in 500 g. If the concentration of salmonellae in the lot is one in 500 g, the probability of finding all sixty 25-g subsamples negative is about 5% (2). It is assumed that the same probability is provided by the other analytical procedures. Thus in seven of eight negative samples, the level of salmonellae was below that which could be detected with 95% confidence. The exception was cottonseed meal used in test 18 in which the estimated *Salmonella* level was 1.32 per 500 grams. This sample was positive in three 100-g subsamples, but all three wet composites were negative. In such a case one or more of the negative wet composites must be considered as "false negative."

Twenty-five *Salmonella* serotypes were obtained from the 17 product-samples tested.

Discussion

Analytical results from 26 samples of naturally contaminated dried foods indicate that *Salmonella*-positive samples may be detected with equal facility by analyzing sixty 25-g subsamples, fifteen 100-g subsamples, or three 500-g subsamples. Pooling sixty 25-g or fifteen 100-g pre-enrichment cultures into groups of five (wet compositing) followed by selective enrichment of the composites apparently results in the same assurance of detection of positive lots as the analysis of individual samples.

Limited data suggest that the analysis of a single 1500-g subsample is feasible. Eleven of the samples were analyzed by pre-enriching a single 1500-g subsample in 13 500 ml of broth. Ten of 11 samples were *Salmonella*-positive with the negative result coming from the material used

TABLE 2

Patterns in the recovery of salmonellae by five different sampling and analysis methods

Sampling and analytical method						
Dry			Wet composites			
Wt. of subsamples, g						
25	100	500	25	100		
No. of subsamples					Samples positive for each pattern	Estimated *Salmonella* level/500 g
60	15	3	12	3		
+*	+	+	+	+	18	
+	+	−†	+	+	1	0.56
+	+	+	+	−	1	1.32
+	−	+	+	−	1	0.56
−	+	+	−	+	2	0.22; 0.22
+	−	−	+	−	1	0.22
−	−	+	−	−	2	0.11; 0.11

*Plus (+) indicates one or more of the subsamples were positive.
†Minus (−) indicates none of the subsamples were positive.

for test 24, diluted fish meal. Failure to isolate *Salmonella* from this 1500-g subsample is probably a "false negative," because the sample had a relatively high level of contamination.

The efficacy of using large samples for *Salmonella* analysis permits statistical quality control which is economically feasible. This is extremely important, because the best way to discover a *Salmonella* problem is through constant surveillance of finished product and the plant environment. In addition, sampling plans being recommended for certain foods require examination of a large number of samples to attain a high probability of safety.

Routine surveillance requires collection and examination of large numbers of samples, especially in a large plant producing many products. If the samples from individual lots can be lumped as described here, the monetary and time savings are obvious. Similarly, substantial savings would result by compositing in the application of strict sampling plans such as those recommended by the NRC, Committee on *Salmonella* (2) and the International Commission on Microbiological Specifications for Foods (report in preparation). For example, the NRC Committee recommended sixty 25-g samples for category I foods (powdered infant foods, therapeutic diet supplements, geriatric food and drugs), which are to be used by populations having high susceptibility to a *Salmonella* infection. The present study indicates that these products could be as effectively controlled by analyzing three 500-g samples per lot. Category II includes high-risk foods which contain a sensitive ingredient (s), have no final pasteurization, and are capable of supporting *Salmonella* growth. The recommended sampling plan requires analysis of twenty-nine 25-g samples for a total of 725 g. The results of this study show that analysis of two 500-g subsamples would permit a higher measure of control over such products than analysis of twenty-nine 25-g samples, since this entails the analysis of an additional 375 g.

Similarly, the less critical products in categories III, IV, and V could be statistically controlled by analyzing a single pool of twenty 25-g (500 g) samples, since the recommended analytical scheme entails the analysis of thirteen 25-g (325 g) samples.

The NRC report directed its attention to the problems of *Salmonella* control in the United States. The difficulties encountered in the control of foods in international commerce are much greater, since it is more likely that the history of a given lot of product is unknown. Here the need for statistical quality control over the *Salmonella* defect is correspondingly greater. At present, cursory or the complete absence of sampling affords little protection to the consumer.

Attribute sampling plans assume random distribution of a defect in a lot. It should be emphasized that in the present studies the sample received for testing was thoroughly mixed in the laboratory to promote random distribution of salmonellae. The question of the proper procedures to be used in obtaining representative material from lots of food for laboratory testing remains moot and was not a part of this investigation.

The foods tested in these experiments were naturally contaminated with salmonellae, and the diversity of *Salmonella* serotypes was limited. Nevertheless, 25 serotypes were isolated from the samples examined, and it appears that the results of the study were not serotype-dependent.

The present study involved dried foods, but similar sampling procedures for high-moisture products, such as meats, poultry, and bulk eggs are being investigated.

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